

## Comparison of Recovery of Blood Culture Isolates from Two BacT/ALERT FAN Aerobic Blood Culture Bottles with Recovery from One FAN Aerobic Bottle and One FAN Anaerobic Bottle

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Traditionally, a routine blood culture for adult patients consisted of paired aerobic and anaerobic bottles, but the routine use of an anaerobic blood culture bottle has been challenged in recent years. In this study, we compared the recovery of two FAN aerobic bottles with one FAN aerobic and one FAN anaerobic bottle. Each pair of bottles was collected by a separate collection procedure, and each bottle held a recommended 8- to 12-ml draw. A total of 704 clinically significant isolates were recovered from 8,620 sets (17,240 pairs), with 487 (69.2%) isolates recovered from one or both bottles in each pair of bottles, 86 isolates (12.2%) recovered only from the FAN aerobic-FAN aerobic pair, and 131 isolates (18.6%) recovered only from the FAN aerobic-FAN anaerobic pair. Significantly more total organisms ( $P = 0.002$ ), gram-positive cocci ( $P = 0.03$ ), *Staphylococcus aureus* ( $P = 0.05$ ), *Enterobacteriaceae* other than *Escherichia coli* ( $P = 0.02$ ), and anaerobes ( $P = 0.01$ ) were recovered from the FAN aerobic-FAN anaerobic pair than from the FAN aerobic-FAN aerobic pair. A separate analysis was performed on the 618 isolates that were recovered from the FAN aerobic-FAN anaerobic pair to compare recovery by bottle type. Significantly more *S. aureus* ( $P = 0.005$ ) and anaerobes ( $P < 0.001$ ) were recovered from the FAN anaerobic bottle, while significantly more coagulase-negative staphylococci ( $P = 0.01$ ), *Streptococcus pneumoniae* ( $P = 0.03$ ), and other gram-negative bacilli ( $P = 0.004$ ) were recovered from the FAN aerobic bottle. These results support the routine use of a FAN anaerobic bottle for use in the culture of blood with the BacT/ALERT system in our institution. These results also suggest that the decision of whether to routinely utilize an anaerobic blood culture bottle should be influenced by the overall recovery of bacteria and yeast, the recovery of specific types of bacteria or yeast, the medium type, and the blood culture system utilized by the laboratory.

Traditionally, in most clinical microbiology laboratories in the United States, a routine blood culture for adults has consisted of paired aerobic and anaerobic blood culture bottles. Depending upon the methodology and bottle type employed, the volume of blood placed into each bottle has generally ranged from 3 to 10 ml for adult patients.

Beginning with a report by Sharp (11), a number of publications have challenged the need for the use of a routine anaerobic blood culture bottle, essentially asking whether the inoculation of the same volume of blood into two aerobic blood culture bottles, at least on a selective basis, would be more clinically beneficial than the use of the more traditionally employed aerobic-anaerobic pair (1, 2, 8, 9, 12).

Implicit in the argument to restrict the use of anaerobic blood culture bottles is the premise that were the same amount of blood inoculated into a second aerobic bottle rather than an anaerobic bottle, the overall yield would be greater with two aerobic bottles than with the aerobic-anaerobic pair. The purpose of this study was to test that hypothesis with the BacT/ALERT blood culture system, comparing the yield of two FAN aerobic blood culture bottles with the yield of one FAN aerobic

bottle and one FAN anaerobic bottle. Importantly, this study was performed utilizing a total recommended 40-ml blood culture draw divided among the four bottles.

### MATERIALS AND METHODS

Most adult patients at Geisinger Medical Center have two blood cultures collected sequentially as a standard of care. A culture consists of two BacT/ALERT blood culture bottles, with each bottle containing a recommended 10 ml of blood. Phlebotomists are instructed to collect each pair of bottles via a separate procedure. When possible, two separate peripheral venipunctures are performed; however, for some patients, one or both sets are collected through a central line.

For this study, sets of four BacT/ALERT FAN blood culture bottles were specially prepared in the laboratory. Each set included two pairs of bottles. One pair consisted of two FAN aerobic bottles while the other pair consisted of one FAN aerobic bottle and one FAN anaerobic bottle. A one or a two was placed on each pair of bottles to facilitate collecting a FAN aerobic-FAN aerobic pair first for approximately one-half of the patients while the FAN aerobic-FAN anaerobic pair was collected first for the remainder of the patient draws. The standard Geisinger Medical Center protocol was followed for skin preparation prior to collection. No attempt was made to verify the actual volume of blood collected per bottle.

For the sake of clarity, the following terminology is consistently utilized in this manuscript: the term "set" refers to all four bottles collected during a patient episode, the term "pair" refers to two bottles collected by a single collection procedure, and the term "bottle" refers to one single bottle. Hence, there are two bottles in a pair and two pairs in a set.

All bottles were incubated in BacT/ALERT blood culture cabinets at 35°C, using a 5-day protocol. Culture workup and identification were performed using the standard Geisinger Medical Center microbiology laboratory procedures.

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TABLE 1. Comparative yield of 704 clinically significant isolates of bacteria and yeasts from paired FAN aerobic-FAN aerobic and FAN aerobic-FAN anaerobic blood culture bottles

| Microorganism(s)                             | No. of isolates from: |                                      |  | P value <sup>a</sup> |
|--|-----------------------|--------------------------------------|--|----------------------|
|  | Both bottle pairs     | FAN aerobic-FAN aerobic bottles only | FAN aerobic-FAN anaerobic bottles only |                      |
| Gram-positive cocci                          | 317                   | 38                                   | 59                                     | 0.03                 |
| <i>S. aureus</i>                             | 139                   | 23                                   | 38                                     | 0.05                 |
| Coagulase-negative <i>Staphylococcus</i>     | 92                    | 0                                    | 0                                      |                      |
| Enterococci <sup>b</sup>                     | 24                    | 8                                    | 13                                     | NS                   |
| <i>S. pneumoniae</i>                         | 24                    | 1                                    | 1                                      | NS                   |
| Other streptococci <sup>c</sup>              | 37                    | 6                                    | 6                                      | NS                   |
| Other gram-positive cocci <sup>d</sup>       | 1                     | 0                                    | 1                                      | NC                   |
| <i>Listeria monocytogenes</i>                | 1                     | 0                                    | 0                                      | NC                   |
| Gram-negative bacilli                        | 154                   | 41                                   | 55                                     | NS                   |
| <i>E. coli</i>                               | 83                    | 21                                   | 26                                     | NS                   |
| Other <i>Enterobacteriaceae</i> <sup>e</sup> | 60                    | 9                                    | 22                                     | 10.02                |
| Other gram-negative bacilli <sup>f</sup>     | 11                    | 11                                   | 7                                      | NS                   |
| Anaerobes <sup>g</sup>                       | 9                     | 5                                    | 16                                     | 0.01                 |
| Yeasts <sup>h</sup>                          | 6                     | 2                                    | 1                                      | NC                   |
| Total (no. [%])                              | 487 (69.2)            | 86 (12.2)                            | 131 (18.6)                             | 0.002                |

<sup>a</sup> NS, not significant ( $P > 0.05$ ); NC, not calculated when total number of isolates was  $\leq 10$ .

<sup>b</sup> Includes 14 *Enterococcus faecium*, 27 *Enterococcus faecalis*, and 4 *Enterococcus* sp. isolates.

<sup>c</sup> Includes 5 group A, 28 group B, 1 group F, 4 group G, and 7 viridans group streptococci, 3 *Streptococcus bovis*, and 1 *S. bovis* var. isolate.

<sup>d</sup> Includes one *Gamella* sp. and one *Abiotrophia* sp. isolate.

<sup>e</sup> Includes 6 *Citrobacter freundii*, 1 *Enterobacter sakazaki*, 6 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 31 *Klebsiella pneumoniae*, 3 *Klebsiella oxytoca*, 17 *Proteus mirabilis*, 1 *Proteus penneri*, 2 *Providencia stuartii*, 4 *Salmonella* spp., 15 *Serratia marcescens*, and 4 *Enterobacter agglomerans* isolates.

<sup>f</sup> Includes 1 *Acinetobacter baumannii*, 1 *Acinetobacter lwoffii*, 16 *Pseudomonas aeruginosa*, 6 *Haemophilus influenzae*, 1 *Neisseria meningitidis*, 1 *Aeromonas* sp., 1 *Campylobacter fetus*, 1 *Stenotrophomonas maltophilia*, and 1 oxidase-positive gram-negative bacillus isolate.

<sup>g</sup> Includes two anaerobic gram-positive cocci, three *Bacteroides fragilis* group, two *B. fragilis*, one *Bacteroides thetaiotaomicron*, three *Bacteroides vulgatus*, one *Bacteroides eggerthii*, one *Bacteroides urealyticus*, one *Fusobacterium nucleatum*, three *Clostridium septicum*, five *Clostridium perfringens*, two *Clostridium* sp., four *Eubacterium lentum*, one *Bifidobacterium* sp., and one *Bacteroides caccae* isolate.

<sup>h</sup> Includes two *Candida albicans*, two *Candida glabrata*, one *Candida guilliermondii*, three *Candida tropicalis*, and one *Candida* sp. (not *albicans*) isolate.

Only bottles which were signaled as positive by the instrument were subcultured. No blind subcultures were performed on any bottles.

For the purposes of this study, isolates of coagulase-negative staphylococci, *Bacillus* spp., viridans group streptococci, *Propionibacterium* spp., and aerobic diphtheroids isolated from only one pair of bottles in a set were considered to be clinically not significant. Statistical analysis was performed as described by Ilstrup (4).

## RESULTS

A total of 8,620 blood culture sets (17,240 pairs; 34,480 bottles) were included in this study. From these 8,620 sets, 1,364 bacterial and yeast isolates were recovered, including 704 judged to be clinically significant and 660 judged to be clinically not significant.

Table 1 summarizes the 704 clinically significant isolates. Of these, 487 (69.2%) isolates were detected in one or both bottles in each pair of bottles, while 86 (12.2%) were detected only in the FAN aerobic-FAN aerobic paired bottles and 131 (18.6%) were detected only in the FAN aerobic-FAN anaerobic paired bottles ( $P = 0.001$ ). Significantly more gram-positive cocci ( $P = 0.03$ ), *Staphylococcus aureus* ( $P = 0.05$ ), *Enterobacteriaceae* other than *Escherichia coli* ( $P = 0.02$ ), and anaerobes ( $P = 0.01$ ) were recovered from the FAN aerobic-FAN anaerobic pair than from the FAN aerobic-FAN aerobic pair.

To better compare the relative recoveries of the FAN aer-

obic and FAN anaerobic bottles, we analyzed the subset of isolates that were recovered from the FAN aerobic-FAN anaerobic pair but not those recovered from the FAN aerobic-FAN aerobic pair. Included in this analysis were the 487 isolates that were detected in both pairs of bottles and the 131 isolates detected only in the FAN aerobic-FAN anaerobic pair (Table 2). Analysis of the recovery of these 618 isolates demonstrated no significant difference in recovery between the FAN aerobic and FAN anaerobic bottles. However, significantly more *S. aureus* ( $P = 0.005$ ) and anaerobes ( $P < 0.001$ ) were recovered from the FAN anaerobic bottles, while significantly more coagulase-negative staphylococci ( $P = 0.01$ ), *Streptococcus pneumoniae* ( $P = 0.03$ ), and other gram-negative bacilli ( $P = 0.004$ ) were recovered from the FAN aerobic bottle.

## DISCUSSION

Most laboratories in the United States have historically utilized paired aerobic and anaerobic bottles for routine blood cultures. The use of the two atmospheres, combined with the same or different types of liquid medium, is intended to maximize the yield of obligate aerobic, obligate anaerobic, and facultative anaerobic microorganisms.

This practice of routine use of paired aerobic and anaerobic

TABLE 2. Comparative yield of 618 clinically significant isolates of bacteria and yeasts from FAN aerobic-FAN anaerobic bottle pair by bottle type

| Microorganism(s)                             | No. of isolates from: |                         |                           | P value <sup>a</sup> |
|--|-----------------------|-------------------------|---------------------------|----------------------|
|  | Both bottles          | FAN aerobic bottle only | FAN anaerobic bottle only |                      |
| Gram-positive cocci                          | 250                   | 60                      | 66                        | NS                   |
| <i>S. aureus</i>                             | 120                   | 18                      | 39                        | 0.005                |
| Coagulase-negative <i>Staphylococcus</i>     | 60                    | 23                      | 9                         | 0.01                 |
| Enterococci <sup>b</sup>                     | 20                    | 5                       | 12                        | NS                   |
| <i>S. pneumoniae</i>                         | 17                    | 7                       | 1                         | 0.03                 |
| Other streptococci <sup>c</sup>              | 33                    | 6                       | 4                         | NS                   |
| Other gram-positive cocci <sup>d</sup>       | 0                     | 1                       | 1                         | NC                   |
| <i>Listeria monocytogenes</i>                | 1                     | 0                       | 0                         | NC                   |
| Gram-negative bacilli                        | 127                   | 48                      | 34                        | NS                   |
| <i>E. coli</i>                               | 70                    | 18                      | 21                        | NS                   |
| Other <i>Enterobacteriaceae</i> <sup>e</sup> | 51                    | 19                      | 12                        | NS                   |
| Other gram-negative bacilli <sup>f</sup>     | 6                     | 11                      | 1                         | 0.004                |
| Anaerobes <sup>g</sup>                       | 9                     | 0                       | 16                        | <0.001               |
| Yeasts <sup>h</sup>                          | 0                     | 7                       | 0                         | NC                   |
| Total (no. [%])                              | 387 (62.6)            | 115 (18.6)              | 116 (18.8)                | NS                   |

<sup>a</sup> NS, not significant ( $P > 0.05$ ); NC, not calculated when total number of isolates was  $\leq 10$ .

<sup>b</sup> Includes 11 *Enterococcus faecium*, 23 *Enterococcus faecalis*, and 3 *Enterococcus* sp. isolates.

<sup>c</sup> Includes 5 group A, 26 group B, 2 group G, and 6 viridans group streptococci, 3 *Streptococcus bovis*, and 1 *S. bovis* var. isolate.

<sup>d</sup> Includes one *Gamella* sp. and one *Abiotrophia* sp. isolate.

<sup>e</sup> Includes 6 *Citrobacter freundii*, 3 *Enterobacter agglomerans*, 2 *Enterobacter aerogenes*, 3 *Enterobacter cloacae*, 1 *Enterobacter sakazaki*, 2 *Klebsiella oxytoca*, 28 *Klebsiella pneumoniae*, 15 *Proteus mirabilis*, 1 *Proteus penneri*, 2 *Providencia stuartii*, 4 *Salmonella* spp., and 15 *Serratia marcescens* isolates.

<sup>f</sup> Includes one *Acinetobacter twofii*, one *Aeromonas* sp., one *Campylobacter fetus*, five *Haemophilus* spp., nine *Pseudomonas aeruginosa*, and one *Stenotrophomonas maltophilia* isolate.

<sup>g</sup> Includes two anaerobic gram-positive cocci, two *Bacteroides fragilis*, two *B. fragilis* group, one *Bacteroides caccae*, one *Bacteroides thetaiotaomicron*, two *Bacteroides vulgatus*, one *Bacteroides eggerthii*, one *Bacteroides urealyticus*, one *Bifidobacterium* sp., three *Clostridium perfringens*, three *Clostridium septicum*, one *Clostridium* sp. (not *perfringens*), four *Eubacterium lentum*, and one *Fusobacterium nucleatum* isolate.

<sup>h</sup> Includes two *Candida albicans*, one *Candida glabrata*, one *Candida guilliermondii*, two *Candida tropicalis*, and one *Candida* sp. (not *Candida albicans*) isolate.

blood culture bottles was challenged in a series of articles which noted a relative decline in the number of isolates of obligate anaerobic bacteria and a concomitant increase in the number of isolates of obligate aerobic or facultative anaerobic bacteria and yeasts (1, 2, 8, 9, 12). Specifically, in 1991, Sharp questioned whether routine anaerobic blood cultures were still appropriate (11). Others have advocated the selective use of anaerobic blood cultures, restricting them to patients with specific illnesses or diseases or undergoing specific procedures such as colorectal or gynecological surgery (1, 2, 8, 9, 12). Indeed, the topic of whether an anaerobic blood culture should routinely be performed was the subject of several articles and editorials in the medical literature (6, 7, 10).

No expert in the field has suggested that the total amount of blood cultured be reduced by the elimination of an anaerobic blood culture bottle. Rather, implicit in the argument that the routine use of anaerobic blood cultures should be limited is the premise that the same volume of blood that is inoculated into an anaerobic bottle, if inoculated instead into an additional aerobic blood culture bottle, would increase the overall yield of significant bacteria and yeasts.

The results of this study would appear to contradict the evidence and opinions of others who have suggested that the yield from two aerobic bottles would be greater than the yield from paired aerobic and anaerobic bottles (5, 8). Ziegler and colleagues asserted that the increase in recovery from the an-

aerobic bottle was due to the increased volume, not the anaerobic atmosphere (15). The results of this study do not support that assertion. There are at least three possible explanations for the differences between the experience and/or speculation of others and the results of this study. These relate to the blood culture system and type of bottle employed, the recovery of specific species or groups of microorganisms, and the demographics of the patients served by the laboratory.

Clearly, conclusions on the appropriateness of routine use of an anaerobic blood culture bottle must be based upon the blood culture method employed by the laboratory and the specific type(s) of liquid medium employed. Cornish and colleagues, in an analysis of the data collected from the use of FAN aerobic and standard BacT/ALERT blood culture bottles, advocated the selective use of an unvented anaerobic bottle based upon patient location and disease service (2). Subsequently, in another study, these same authors compared the performance of standard BacT/ALERT anaerobic blood culture bottles with the FAN anaerobic blood culture bottles (3). They reported that while the FAN aerobic bottle recovered more *Pseudomonas* and yeasts than the FAN anaerobic bottle, there were no differences in the ability of the two bottles to detect episodes of bacteremia. Furthermore, they noted no differences in recovery of *Enterobacteriaceae* between the two bottles. Lastly, they noted that the incidence of anaerobic bacteremia, which was less than 1% in the late 1980s and early

1990s in their institution, had now increased to 5.8%. Whether this was due to changing patient demographics or changes in blood culture methodology was not clear to these authors.

As far as we are aware, none of the other studies that have advocated selective use of anaerobic culture have been based upon data accumulated from the use of FAN aerobic and FAN anaerobic media. Weinstein and colleagues showed significantly greater recovery of microorganisms from the BacT/ALERT FAN aerobic bottle than from the standard BacT/ALERT aerobic bottle (13). Perhaps of particular relevance to this study, Wilson and colleagues reported significantly greater recovery of microorganisms from the BacT/ALERT FAN anaerobic bottle than from the BacT/ALERT standard anaerobic bottle (14). It is therefore reasonable to assert that speculation on the benefit of a BacT/ALERT standard anaerobic blood culture bottle is not necessarily applicable to the benefit of the use of a BacT/ALERT FAN anaerobic blood culture bottle. Furthermore, differences in headspace gas volume and composition as well as media formulations between various bottle types likely influence the recovery of specific microorganisms.

The second factor that clearly influences the recovery of bacteria and yeasts from an aerobic and anaerobic bottle is organism related. When the overall yields of the paired bottles in this study are compared (Table 1), the FAN aerobic-FAN anaerobic pair recovered significantly more bacteria and yeasts than the FAN aerobic-FAN aerobic pair. Yet when all of the isolates recovered from the FAN aerobic-FAN anaerobic pair are analyzed by bottle type, there is no significant difference in total organisms recovered (Table 2). Thus, there is an apparent contradiction in the results of this study as presented in Tables 1 and 2. A more careful examination of the data offers a possible explanation for this apparently paradoxical result. Table 1 summarizes recovery from 20 ml of blood from paired bottles, while Table 2 summarizes recovery from 10 ml of blood from one bottle. If an organism is more easily detected, either due to a higher concentration of organisms in the blood or to the blood culture methodology (including medium composition), the percentage of recovery in the first bottle can be very high, making the recovery in the second bottle (and, with that, the atmosphere) less important. An example of this in this study is a comparison of the recovery of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Twenty-four isolates (92.3%) of *Streptococcus pneumoniae* were recovered from both pairs of bottles, with a single isolate recovered from each of the paired bottles. Yet when isolation by bottle type was examined (Table 2), 24 of 25 isolates were recovered from the aerobic bottle and 18 of 25 were recovered from the anaerobic bottle. We submit, therefore, that for *Streptococcus pneumoniae*, the choice of the second bottle type does not influence overall yield. On the other hand, 139 of 200 (69.5%) *Staphylococcus aureus* isolates were recovered from both pairs of bottles, with 23 (11.5%) recovered from only the FAN aerobic-FAN aerobic pair and 38 (19%) recovered from only the FAN aerobic-FAN anaerobic pair. When isolation by bottle type was examined, 138 of 177 (80%) isolates were recovered from the FAN aerobic bottle and 159 of 177 (89.8%) were recovered from the FAN anaerobic bottle. Therefore, we submit that an examination of differences in total yield as presented in Table 1 is more important than the relative recovery by bottle type as presented in Table 2. Thus, the relative mixture of organisms

isolated in a particular institution has a direct influence on the relative benefit of the anaerobic bottle.

The third factor that clearly influences the recovery of bacteria and yeasts is the patient demographics of the particular institution. Geisinger Medical Center is a tertiary care medical center with oncology and transplant services, but the transplant program is limited to renal transplants and autologous bone marrow transplants. Of the 704 clinically significant isolates recovered in this study, there were a total of 8 yeast and 16 *Pseudomonas aeruginosa* isolates. Institutions with large numbers of solid organ transplants and/or larger numbers of oncology patients might anticipate proportionately larger numbers of opportunistic infections with *Pseudomonas* spp. and yeasts. Therefore, we would anticipate that a study similar to ours but performed in an institution with different patient demographics from ours might, indeed, yield different results.

In conclusion, the results of this study support the continued, routine use of a FAN anaerobic bottle in conjunction with a FAN aerobic bottle for use in the culture of blood with the BacT/ALERT system in our institution. We were able to recover significantly more microorganisms from the FAN aerobic-FAN anaerobic pair than from the FAN aerobic-FAN aerobic pair, indicating that the benefit of the FAN anaerobic bottle goes beyond what might have been expected merely from the culture of an additional 8 to 12 ml of blood. These results also suggest that the decision of whether to routinely utilize an anaerobic blood culture bottle should be influenced by the overall recovery of bacteria and yeasts as well as the recovery of specific types of bacteria or yeast.

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